



US009376667B2

(12) **United States Patent**
Yoshida et al.(10) **Patent No.:** US 9,376,667 B2
(45) **Date of Patent:** Jun. 28, 2016(54) **PROTEIN HAVING NADH AND/OR NADPH
OXIDASE ACTIVITY**
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WO WO-2006/090814 A1 8/2006
WO WO-2008/066018 A1 6/2008(73) Assignee: **Kaneka Corporation**, Osaka (JP)
(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.(21) Appl. No.: **15/011,989**(22) Filed: **Feb. 1, 2016**(65) **Prior Publication Data**

US 2016/0145585 A1 May 26, 2016

Related U.S. Application Data(62) Division of application No. 13/574,458, filed as
application No. PCT/JP2011/050824 on Jan. 19, 2011.(30) **Foreign Application Priority Data**

Jan. 20, 2010 (JP) 2010-010308

(51) **Int. Cl.**
C12N 9/02 (2006.01)(52) **U.S. Cl.**
CPC **C12N 9/0036** (2013.01); **C12Y 106/03**
(2013.01)(58) **Field of Classification Search**
CPC C12Y 106/03001
See application file for complete search history.(56) **References Cited**

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(57) **ABSTRACT**Water-forming NADH oxidase derived from *Streptococcus
mutans* should be further improved in terms of stability for
practical use in industrial production. An object of the present
invention is to provide an enzyme that is obtained through
modification of a water-forming NADH oxidase, which is
useful as an NAD⁺ regeneration system for stereoselective
oxidation catalyzed by an oxidoreductase, by protein engi-
neering techniques so that the enzyme can withstand long-
term use without exhibiting a reduction of its activity for the
regeneration of NAD⁺, that is, an enzyme having improved
stability, and to provide a method for efficiently producing a
useful substance such as an optically active alcohol or amino
acid. The present invention relates to an enzyme modification
method that can improve the stability of water-forming
NADH oxidase derived from *Streptococcus mutans* by appro-
priately introducing mutation.**6 Claims, No Drawings**

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**PROTEIN HAVING NADH AND/OR NADPH
OXIDASE ACTIVITY**

CROSS REFERENCE TO RELATED
APPLICATIONS

This application is a Divisional application of U.S. application Ser. No. 13/574,458 filed on Jul. 20, 2012, which is a National Phase filing under 35 U.S.C. §371 of PCT/JP2011/050824 filed on Jan. 19, 2011; and this application claims priority to Application No. 2010-010308 filed in Japan on Jan. 20, 2010 under 35 U.S.C. §119; the entire contents of all are hereby incorporated by reference.

TECHNICAL FIELD

The present invention relates to NAD(P)H oxidase variants.

BACKGROUND ART

Reactions that involve oxidoreductases activated by the coenzyme nicotinamide adenine dinucleotide to synthesize compounds of interest are widely used in industrial processes. Many of these compounds of interest are optically active compounds which are mainly produced as precursors of medicaments and agricultural chemicals (Non Patent Literatures 1 and 2). The redox reactions involving oxidoreductases are accompanied with either the conversion of NAD(P)⁺ (oxidized coenzyme) into NAD(P)H (reduced coenzyme) or the reverse conversion of NAD(P)H into NAD(P)⁺. Therefore, these redox reactions require a stoichiometric amount of NAD(P)⁺ or NAD(P)H. In industrial processes, it is preferable to avoid the use of a stoichiometric amount of such an expensive coenzyme. In this context, a technique that can reduce the amount of the expensive coenzyme has been used in industrial fields, in which the redox reaction is coupled with the conversion of the coenzyme formed as a result of the redox reaction into the form reusable for the reaction (oxidized form or reduced form) (Non Patent Literatures 2 and 3).

NAD(P)H oxidases are one of oxidoreductases that can be used for the conversion of NAD(P)H into NAD(P)⁺. Oxidation reactions of alcohols, amino acids, and the like which are catalyzed by nicotinamide coenzyme-dependent oxidoreductases utilize NAD(P)⁺ and produce NAD(P)H. NAD(P)H oxidases, which catalyze the conversion of NAD(P)H into NAD(P)⁺, can be involved in the oxidation of alcohols, amino acids, and the like, as an enzyme for regenerating NAD(P)⁺ (as a second enzyme system) (Patent Literatures 1 to 3 and Non Patent Literatures 3 and 4).

Well-known NAD(P)H oxidases used for industrial purposes are ones that produce a by-product such as hydrogen peroxide (H₂O₂) or water (H₂O) as a result of reduction of molecular oxygen which occurs simultaneously with the oxidation of NAD(P)H to NAD(P)⁺ (Non Patent Literatures 3 and 4). Water-forming NAD(P)H oxidases are suitable for the NAD(P)⁺ regeneration system since they irreversibly catalyze the production of NAD(P)⁺ from NAD(P)H. H₂O₂-forming NAD(P)H oxidases are not easily used for enzyme-involved chemical reaction processes because produced H₂O₂ is toxic to enzymes. Therefore, ones that produce only water as a reaction product in addition to NAD(P)⁺ are preferred for industrial purposes.

Examples of known water-forming NAD(P)H oxidases include NADH oxidase derived from *Lactobacillus brevis*, NADH/NADPH (both can be substrates) oxidase derived from *Lactobacillus sanfranciscensis*, NADH oxidase derived

from *Pyrococcus furiosus*, and NADH oxidase derived from *Borrelia burgdorferi* (Non Patent Literatures 4 to 6). Methods for synthesizing an optically active compound (optical resolution) have been proposed which utilize such a water-forming NAD(P)H oxidase as an NAD(P)⁺ regeneration system (Patent Literature 1 and Non Patent Literatures 5 to 8).

Water-forming NADH oxidases derived from bacteria of *Streptococcus*, in particular *Streptococcus mutans*, are also known (Patent Literature 2 and Non Patent Literatures 9 to 11). It has already been verified that these enzymes can be used as second enzyme systems for regenerating NAD(P)⁺ in oxidation reactions of alcohols, amino acids, and the like which are catalyzed by nicotinamide coenzyme-dependent oxidoreductases (Patent Literatures 3 and 4). These enzymes are characteristically known to efficiently catalyze the regeneration of NAD⁺ in the absence of enzyme stabilizers such as reductants (Patent Literature 2). This is a superior characteristic in terms of industrial usability, compared with other NAD(P)H oxidases such as NADH oxidase derived from *Lactobacillus brevis* which require an additive such as DTT (dithiothreitol) (Non Patent Literature 5).

CITATION LIST

Patent Literature

Patent Literature 1: JP-A 2003-116585
Patent Literature 2: JP-A H08-196281
Patent Literature 3: WO 06/013802
Patent Literature 4: WO 06/033333

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SUMMARY OF INVENTION

Technical Problem

Water-forming NADH oxidase derived from *Streptococcus mutans* should be further improved in terms of safety for practical use in industrial production. Specifically, it has been found to have a disadvantage in that the enzyme activity for the regeneration of NAD⁺ remarkably reduces with time. An object of the present invention is to provide an enzyme that is obtained through modification of a water-forming NADH

oxidase, which is useful as an NAD⁺ regeneration system for stereoselective oxidation catalyzed by an oxidoreductase, by protein engineering techniques so that the enzyme can withstand long-term use without exhibiting a reduction of its activity for the regeneration of NAD⁺, that is, an enzyme having improved stability, and to provide a method for efficiently producing a useful substance such as an optically active alcohol or amino acid.

Solution to Problem

As a result of intensive studies to overcome the above problem, the present inventors have developed a novel enzyme modification method that can improve the stability of water-forming NAD(P)H oxidase derived from *Streptococcus mutans*. This method can therefore be employed as an enzyme modification method for improving the stability of NAD(P)H oxidases which have high sequence identity thereto.

Specifically, the present invention relates to the following.

[1] A protein having an amino acid sequence that has at least 85% sequence identity to the amino acid sequence of SEQ ID No:1, and further contains at least one amino acid substitution selected from (o) to (u):

(o) a substitution of an amino acid residue at a position conformationally equivalent to Leu-42 with an amino acid having a side-chain surface area of 100 to 200 (Å²);

(p) a substitution of an amino acid residue at a position conformationally equivalent to Met-46 with a neutral amino acid having a side-chain surface area of 100 to 150 (Å²) or an acidic amino acid having a side-chain surface area of 100 to 150 (Å²);

(q) a substitution of an amino acid residue at a position conformationally equivalent to Asn-96 with a basic amino acid;

(r) a substitution of an amino acid residue at a position conformationally equivalent to Tyr-172 with an amino acid having a smaller side-chain surface area than Tyr;

(s) a substitution of an amino acid residue at a position conformationally equivalent to Thr-196 with a basic amino acid;

(t) a substitution of an amino acid residue at a position conformationally equivalent to Ala-312 with an amino acid having a larger side-chain surface area than Ala; and

(u) a substitution of an amino acid residue at a position conformationally equivalent to Phe-371 with an aliphatic amino acid, an acidic amino acid, or an amino acid having a hydroxyl group-bearing side chain.

[2] The protein defined in [1], wherein the amino acid sequence contains at least one amino acid substitution selected from (v) to (bb):

(v) a substitution of an amino acid residue at a position conformationally equivalent to Leu-42 with Met;

(w) a substitution of an amino acid residue at a position conformationally equivalent to Met-46 with Ile;

(x) a substitution of an amino acid residue at a position conformationally equivalent to Asn-96 with Arg or His;

(y) a substitution of an amino acid residue at a position conformationally equivalent to Tyr-172 with Ala or Ser;

(z) a substitution of an amino acid residue at a position conformationally equivalent to Thr-196 with His;

(aa) a substitution of an amino acid residue at a position conformationally equivalent to Ala-312 with Ile; and

(bb) a substitution of an amino acid residue at a position conformationally equivalent to Phe-371 with Ala, Val, Ile, Glu, Ser, Thr, or Tyr.

[3] A protein including an amino acid sequence of SEQ ID No:1 which further contains at least one amino acid substitution selected from (a) to (g):

(a) a substitution of Leu-42 with an amino acid having a side-chain surface area of 100 to 200 (Å²);

(b) a substitution of Met-46 with a neutral amino acid having a side-chain surface area of not more than 150 (Å²) or an acidic amino acid having a side-chain surface area of not more than 150 (Å²);

(c) a substitution of Asn-96 with a basic amino acid;

(d) a substitution of Tyr-172 with an amino acid having a smaller side-chain surface area than Tyr;

(e) a substitution of Thr-196 with a basic amino acid;

(f) a substitution of Ala-312 with an amino acid having a larger side-chain surface area than Ala; and

(g) a substitution of Phe-371 with an aliphatic amino acid, an acidic amino acid, or an amino acid having a hydroxyl group-bearing side chain.

[4] The protein defined in [3], wherein the amino acid sequence contains at least one amino acid substitution selected from (h) to (n):

(h) a substitution of Leu-42 with Met;

(i) a substitution of Met-46 with Ile;

(j) a substitution of Asn-96 with Arg or His;

(k) a substitution of Tyr-172 with Ala or Ser;

(l) a substitution of Thr-196 with His;

(m) a substitution of Ala-312 with Ile; and

(n) a substitution of Phe-371 with Ala, Val, Ile, Glu, Ser, Thr, or Tyr.

[5] The protein defined in claim 4,

wherein the protein has an amino acid sequence selected from the amino acid sequences of SEQ ID Nos:2 and 4 to 19.

[6] A DNA encoding a protein defined in any one of [1] to [5].

[7] A vector containing the DNA defined in [6].

[8] A transformant obtained by transformation with the vector defined in [7].

[9] A culture of the transformant defined in [8].

[10] An enzyme variant-containing product obtained by processing the culture defined in [9].

[11] A method for converting NADH/NADPH (a reduced form) to NAD⁺/NADP⁺ (an oxidized form) using the protein defined in any one of [1] to [5].

[12] The method defined in [11],

wherein the NADH/NADPH (the reduced form) is produced in a reaction catalyzed by an oxidoreductase with nicotinamide adenine dinucleotide as a coenzyme.

[13] The method defined in [11] or [12],

wherein the method utilizes the transformant defined in [8], the culture of the transformant defined in [9], or the enzyme variant-containing product defined in [10].

[14] A compound produced by the method defined in any one of [11] to [13].

[15] The method defined in [12] or [13],

wherein the reaction catalyzed by an oxidoreductase with nicotinamide adenine dinucleotide as a coenzyme is selective oxidation of one enantiomer.

[16] An optically active compound having a high enantiomeric excess, produced by the method defined in [15].

Advantageous Effects of Invention

The NADH oxidases or NADPH oxidases having improved stability according to the present invention can withstand long-term use without exhibiting a reduction of its activity for the regeneration of NAD⁺ or NADP⁺, and therefore efficiently allows the regeneration to proceed. If this

coenzyme regeneration system is coupled with stereoselective oxidation catalyzed by an oxidoreductase, an optically active compound having a high enantiomeric excess can then be efficiently obtained from an enantiomer mixture.

DESCRIPTION OF EMBODIMENTS

The polypeptide of SEQ ID No: 1 is water-forming NADH oxidase derived from *Streptococcus mutans* NCIB11723. The amino acid sequence thereof and the DNA base sequence encoding this have already been known (Patent Literature 2).

The water-forming NADH oxidase is an oxidoreductase that oxidizes the reduced coenzyme NADH to the oxidized coenzyme NAD⁺ and concomitantly uses molecular oxygen as an electron receptor to produce water, as described above. The reaction for producing NAD⁺ from NADH catalyzed by the water-forming NADH oxidase is irreversible, and produces only water as a reaction product other than NAD⁺.

Mutations that may be introduced into the amino acid sequence of SEQ ID No:1 are designed based on three concepts: (I) appropriate protection of the thiol group of the cysteine residue at the catalytic active site from contact with molecular oxygen; (II) removal of the steric hindrance of the NADH-binding site; and (III) contribution to stabilization of the three-dimensional structure of the enzyme in terms of free energy, and are basically embraced within the scope of the present invention as long as the introduced mutation(s) produce one or more of the effects. The following description is offered to illustrate the concepts for the design of mutations in detail.

(I) The mutation (s) for appropriately protecting the thiol group of the cysteine residue at the catalytic active site from contact with molecular oxygen mean that the mutation(s) can adequately prevent molecular oxygen from contacting Cys-44, which is thought to be the catalytic active site (active center) of water-forming NADH oxidase derived from *Streptococcus mutans*, so as to inhibit excessive oxidation of the thiol group.

Specifically, in order to inhibit excessive oxidation by molecular oxygen, the mutation(s) are designed such that a pocket of the catalytic active site to which molecular oxygen will be bound (space which allows molecular oxygen to come closer to the catalytic active site) is narrowed to reduce the rate of the elementary process of molecular oxygen entry. A three-dimensional structure model of the enzyme provides a three-dimensional understanding of the three-dimensional structure of a region around the catalytic active site, in particular, the pocket space. This understanding effectively helps to determine appropriate amino acid mutations and appropriate sites for introducing the mutations to adequately reduce the pocket space and therefore helps to design useful amino acid mutations.

(II) The mutation(s) for removing the steric hindrance of the NADH-binding site mean that the mutation(s) can cause a change of the kinetics of binding and dissociation between NADH and the enzyme which advantageously contributes to stabilization of the enzyme.

On the other hand, crystallographic structural analysis of apoenzymes to which NAD(P)H is not bound has revealed that oxidoreductases (flavoproteins) that utilize FAD and NAD(P)H have three-dimensional structures in which a pocket to which the nicotinamide coenzyme NAD(P)H will be bound (near the isoalloxazine ring of FAD) is shielded by an aromatic amino acid (Carrillo, N. & Ceccarelli, E. A., Eur. J. Biochem. 270, 1900-1915 (2003)). This mechanism of shielding the NAD(P)H-binding pocket by an aromatic ring is generally presumed to function to influence other secondary

reactions (e.g. binding to other molecules) when the shield is removed (i.e. the aromatic ring is considerably shifted). However, water-forming NADH oxidase derived from *Streptococcus mutans* has not been analyzed for such a mechanism in detail yet. In addition, for its industrial purposes, the influence on other secondary reactions does not have to be taken into account.

Then, a substitution of such an aromatic amino acid residue serving as a shield is expected to result in a change of the kinetics of binding and dissociation between NADH and the enzyme, so as to advantageously contribute to stabilization of the enzyme. From a three-dimensional structure model of the enzyme, since the aromatic amino acid residue serving as a shield is presumed to be Tyr-172, mutation designed to reduce the side chain size is considered to be effective.

(III) The mutation(s) that contribute to stabilization of the three-dimensional structure of the enzyme in terms of free energy mean that the mutation(s) can be designed to achieve higher stability of the enzyme based on comparisons of free energy differences between the wild-type and variants.

Specifically, a molecular structure model (the framework of the main chain) can be used to calculate the free energy difference observed with a shift from a denatured state to the native state by molecular simulation calculation (energy minimization calculation) based on molecular mechanics. If the free energy difference is advantageous to the native state, the thermodynamic stability is also high. More specifically, free energy differences between the wild-type and various variants can be calculated by computational screening using the program Shrike (JP 2001-184381 A), and amino acid mutation candidates can then be designed based on the effect of each amino acid substitution on the free energy difference.

In the present invention, the design of "mutation(s)" can be accomplished by using a three-dimensional structure model of water-forming NADH oxidase derived from *Streptococcus mutans* which is constructed by a three-dimensional modeling method. It should be noted that the enzyme of SEQ ID No:1 has not been examined yet by structural analysis such as X-ray crystallographic structural analysis, and therefore its three-dimensional structure remains unknown.

Specifically, first, multiple amino acid sequence alignments of the enzyme and enzymes which have high amino acid sequence homology with the amino acid sequence of the former enzyme and whose three-dimensional structures are registered in the Protein Data Bank (PDB) are constructed using the program ClustalX (Thompson, J. D. et al., Nucleic Acid Res. 22, 4673-80 (1994)). The proteins having high amino acid sequence homology with the enzyme can be selected by amino acid sequence homology search among amino acid sequences of proteins registered in PDB using the program BLAST (Altschul, Stephen F. et al., Nucleic Acids Res. 25, 3389-3402 (1997)) or PSI-BLAST (Shaffer, A. A. et al., Bioinformatics 16, 88-489 (2000)).

Next, three-dimensional structural alignment is performed on these proteins whose three-dimensional structures are known by using a three-dimensional graphics program such as Swiss-PDB Viewer (Guex, N. & Peitsch, M. C., Electrophoresis, 18, 2714-2723 (1997)) and a three-dimensional structure comparison/similar structure search server such as VAST Search (Gibrat, J. F., et al., Curr Opin Struct Biol 6, 377 (1996)). The multiple alignments obtained beforehand based on the amino acid sequences alone are modified based on the similarity between the three-dimensional structures, and then a protein presumed to have a highly similar three-dimensional structure is selected as a template protein for molecular modeling, based on the resulting sequence alignments.

Thus, the three-dimensional structure of its complex with the coenzyme (PDB code: 2NPX) is selected as a template protein for molecular modeling. This template protein is displayed on the program Swiss PDB-Viewer, and subjected to substitution of amino acid residues to correspond to the amino acid sequence (SEQ ID No:1) of the enzyme, based on the sequence alignments. The inserted and deleted sites are replaced with the most suitable similar substructures which are searched from PDB, whereby a three-dimensional structure model can be constructed.

Based on these concepts, mutations each involving at least one selected from amino acid substitutions at positions 42, 46, 96, 172, 196, 312, and 371 of the amino acid sequence of SEQ ID No:1 were designed.

It should be noted that amino acids used for the substitutions are basically selected from 20 proteinogenic amino acids but are intended to include non-proteinogenic amino acids and non-natural amino acids, provided that these substitutions are expected to produce the same effects as those of the later-described amino acid substitutions. Mutations at the corresponding sites accomplished by insertion, deletion, and modification are also encompassed, provided that they are expected to produce the same effects as those of the later-described amino acid substitutions. For example, introducing a deletion at position 45 and an insertion at position 47 together can result in substitution of Met at position 46 with Ala and therefore is expected to produce the same effect as that of the later-described amino acid substitution at position 46.

Specifically, amino acid substitutions that may be introduced into the amino acid sequence of SEQ ID No:1 are the following mutations (1) to (7).

(1) A substitution of Leu-42 with an amino acid that appropriately protects the thiol group of the cysteine residue at the catalytic active site from contact with molecular oxygen. Specifically, it is a substitution with an amino acid having a side-chain surface area of 100 to 200 (\AA^2), and is preferably a substitution with Val (117 \AA^2), Ile (140 \AA^2), Thr (102 \AA^2), Met (160 \AA^2), Asn (113 \AA^2), Gln (144 \AA^2), Asp (106 \AA^2), or Glu (138 \AA^2). More preferably, it is a substitution with Met because it also advantageously contributes to stabilization in terms of free energy. The numbers in parentheses refer to the side-chain surface areas of the respective amino acids.

(2) A substitution of Met-46 with an amino acid that appropriately protects the thiol group of the cysteine residue at the catalytic active site from contact with molecular oxygen. Specifically, it is a substitution with a neutral amino acid having a side-chain surface area of 100 to 150 (\AA^2) or an acidic amino acid having a side-chain surface area of 100 to 150 (\AA^2), and is preferably a substitution with Val (117 \AA^2), Leu (137 \AA^2), Ile (140 \AA^2), Thr (102 \AA^2), Asp (106 \AA^2), or Glu (138 \AA^2). More preferably, it is a substitution with Ile because it also advantageously contributes to stabilization in terms of free energy.

(3) A substitution of Asn-96 with an amino acid that contributes to stabilization of the three-dimensional structure of the enzyme in terms of free energy. Specifically, it is a substitution with a basic amino acid, and is preferably a substitution with Lys, Arg, or His. More preferably, it is a substitution with Arg or His because they provide particularly good energy values based on calculation.

(4) A substitution of Tyr-172 with an amino acid that removes the steric hindrance of the NADH-binding site. Specifically, it is a substitution with an amino acid having a smaller side-chain surface area than Tyr (187 \AA^2), and is preferably a substitution with Gly (0 \AA^2), Ala (67 \AA^2), Val (117 \AA^2), Leu (137 \AA^2), Ile (140 \AA^2), Ser (80 \AA^2), Thr

(102 \AA^2), Asn (113 \AA^2), Gln (144 \AA^2), Asp (106 \AA^2), Glu (138 \AA^2), His (151 \AA^2), Lys (167 \AA^2), or Phe (175 \AA^2). More preferably, it is a substitution with Ala or Ser because they also contribute to stabilization in terms of free energy.

(5) A substitution of Thr-196 with an amino acid that contributes to stabilization of the three-dimensional structure of the enzyme in terms of free energy. Specifically, it is a substitution with a basic amino acid, and is preferably a substitution with Lys, Arg, or His. More preferably, it is a substitution with His because it provides a particularly good energy value based on calculation.

(6) A substitution of Ala-312 with an amino acid that appropriately protects the thiol group of the cysteine residue at the catalytic active site from contact with molecular oxygen. Specifically, it is a substitution with an amino acid having a larger side-chain surface area than Ala (67 \AA^2), and is preferably a substitution with Val (117 \AA^2), Leu (137 \AA^2), Ile (140 \AA^2), Thr (102 \AA^2), Asn (113 \AA^2), Gln (144 \AA^2), Asp (106 \AA^2), Glu (138 \AA^2), His (151 \AA^2), Lys (167 \AA^2), Arg (196 \AA^2), Met (160 \AA^2), Phe (175 \AA^2), Tyr (187 \AA^2), or Trp (217 \AA^2). More preferably, it is a substitution with Val or Ile because they also advantageously contribute to stabilization in terms of free energy.

(7) A substitution of Phe-371 with an amino acid that contributes to stabilization of the three-dimensional structure of the enzyme in terms of free energy. Specifically, it is a substitution with an aliphatic amino acid, an acidic amino acid, or an amino acid having a hydroxyl group-bearing side chain, and is preferably a substitution with Ala, Val, Leu, Ile, Asp, Glu, Ser, Thr, or Tyr. More preferably, it is a substitution with Ala, Val, Ile, Glu, Ser, Thr, or Tyr because they provide particularly good energy values based on calculation.

Amino acids having an acidic side chain are referred to as "acidic amino acids", amino acids having a basic side chain are referred to as "basic amino acids", and the other amino acids are referred to as "neutral amino acids". Based on their isoelectric points (pI), amino acids having a pI of 4.0 or lower are referred to as "acidic amino acids", and amino acids having a pI of 7.0 or higher are referred to as "basic amino acids". The proteinogenic amino acids are categorized as follows based on their isoelectric points (Barrett G. C., "Chemistry and Biochemistry of the Amino Acids", Chapman and Hall, 1985, p. 9, Table 2.2): Asp and Glu are categorized as acidic amino acids; His, Lys, and Arg are categorized as basic amino acids; and the others are categorized as neutral amino acids.

The term "aliphatic amino acid" refers to one whose side chain is a non-cyclic carbon chain. Ala, Val, Leu, and Ile among the proteinogenic amino acids are encompassed therein.

Some of the mutations in the present invention are intended to change the side chain size so as to modify the space in the structure of the protein, and therefore the side-chain surface area is used as a design parameter. The "side-chain surface area" refers to the surface area of the side chain (contactable surface area) which accurately reflects the side chain size, and the side-chain surface area of various amino acids and the particular values are available from known information, for example, in Miller, S., "J. Mol. Biol.", 1987, 196, pp. 641-656 (the particular values of the side-chain surface area are shown in Table 2, for example).

The protein of the present invention is most preferably a protein having an amino acid sequence selected from the amino acid sequences of SEQ ID Nos:2 and 4 to 19 because they provide proteins having particularly high potential in terms of stability and activity.

The mutations in the present invention can be designed by using a three-dimensional structure model of water-forming NADH oxidase derived from *Streptococcus mutans* which is constructed by a three-dimensional modeling method, as described above. Since it is thus easy to apply the present invention to amino acid sequences having sequence identity of at least 85%, preferably at least 90%, more preferably at least 95%, and still more preferably at least 98% to the amino acid sequence of SEQ ID No:1, proteins obtained by introducing mutation(s) usable in the present invention into these amino acid sequences are also included in the scope of the present invention.

The "sequence identity" herein can be determined by amino acid sequence homology analysis using the program BLAST (Altschul, Stephen F. et al., *Nucleic Acids Res.* 25, 3389-3402 (1997)). For BLAST analysis, software available from National Center for Biotechnology Information and the like may be used.

The term "conformationally equivalent position" herein refers to a position that can be readily and objectively identified by amino acid sequence alignment based on the three-dimensional structures of the amino acid sequence of interest and amino acid sequences whose three-dimensional structures are known (e.g. the amino acid sequence with PDB code: 2NPX used in the present invention) using a three-dimensional structure comparison/similar structure search server such as VAST Search. The VAST Search is also available from National Center for Biotechnology Information.

Enzymes having an amino acid sequence that has sequence identity of at least 85% to that of the water-forming NADH oxidase derived from *Streptococcus mutans* (SEQ ID No:1) include NADPH oxidases (or oxidases using NADH or NADPH as a substrate). This is because it is known that even a slight difference in the amino acid sequence of the coenzyme-binding site between enzymes may cause (and can be designed to cause) a difference in the coenzyme selectivity between the enzymes (Penning T. M. & Jez J. M., *Chem. Rev.*, 101, 3027-3046 (2001)), and therefore proteins obtained by applying the present invention are included in the scope of the present invention even if they are not NADH oxidases but NADPH oxidases.

Characteristically, water-forming NAD(P)H oxidase variants obtained by the present invention have the same enzyme activity (function) as that of the wild-type but have more improved stability than the wild-type.

Proteins including an amino acid sequence of SEQ ID No:1 which further contains at least one of the above amino acid substitutions (1) to (7) according to the present invention are essentially NADH oxidase variants characteristically having improved stability compared with the wild-type NADH oxidase having the amino acid sequence of SEQ ID No:1.

The term "improved stability" means that the remaining enzyme activity (%) of a composition containing an enzyme (hereinafter, the term "enzyme" is intended to include enzyme "variants" that maintain enzyme activity) after treatment for a predetermined period of time at a constant temperature is increased compared with an enzyme for comparison subjected to the same treatment. Examples of such treatment include, but are not limited to, storage at rest at a constant temperature (incubation) and agitation with aeration. In the present invention, the remaining enzyme activity after the treatment is calculated based on the enzyme activity (NADH oxidation activity) before the treatment which is taken as 100%.

If the remaining enzyme activity of an NADH oxidase variant of the present invention is increased compared with the remaining enzyme activity of the wild-type NADH oxidase,

the produced NADH oxidase variant is determined to have improved stability. Under a treatment condition which reduces the remaining enzyme activity of the wild-type NADH oxidase to 10 to 40%, the remaining enzyme activity of the NADH oxidase variant having improved stability is higher than the remaining enzyme activity of the wild-type NADH oxidase by 10% or more, preferably by 20% or more, and more preferably by 30% or more. Although not particularly limited, one Unit is defined as the enzyme activity that oxidizes 1 μmol of NADH to NAD⁺ for one minute (the composition and enzyme concentration of a reaction liquid are adjusted to be the same before and after the treatment), and the NADH oxidation activity is calculated based on this definition. The treatment condition is desirably, but is not limited to, agitation with aeration in a solution with such a pH near the neutral pH as a pH of 4.0 to 10.0, preferably a pH of 5.0 to 9.0, at a constant temperature in the range of 4° C. to 80° C., preferably 15° C. to 50° C., for a predetermined period of time.

A DNA encoding the protein of the present invention can be obtained by introducing site-specific mutation(s) into the wild-type NADH oxidase DNA by a recombinant DNA technique, a PCR technique, or the like, as described below.

Specifically, a recombinant DNA technique for introducing mutation(s) is performed as follows. For example, if the wild-type water-forming NADH oxidase gene includes suitable restriction enzyme recognition sequences on both sides of a target site into which a mutation is to be introduced, these sequences are cleaved by the corresponding restriction enzymes to remove the region including the mutation target site, and a DNA fragment containing the mutation only at the target site, which can be prepared by chemical synthesis or the like, is inserted by cassette mutagenesis.

Alternatively, introduction of site-specific mutation(s) by PCR can be performed as follows. One of the ends of the wild-type water-forming NADH oxidase gene is amplified using a mutation primer containing a target mutation at a mutation target site of the wild-type water-forming NADH oxidase gene and a primer for amplification containing the sequence of that one end of the gene without mutations. The other end is amplified using another mutation primer having a complementary sequence to the former mutation primer and another primer for amplification containing the sequence of that other end of the gene without mutations. These two amplified fragments are annealed and subjected to PCR with the two primers for amplification.

The vector of the present invention can be obtained by linking (inserting) the aforementioned water-forming NADH oxidase variant DNA to an appropriate vector.

The vector into which the gene is to be inserted is not particularly limited, provided that it is self-replicable in host cells. Examples of such vectors include plasmid DNAs and phage DNAs. Specific examples of vectors for *E. coli* hosts include plasmid DNAs such as pBR322, pUC18, and pBlue-script II, and phage DNAs such as EMBL3, M13, and $\lambda\text{gt}11$; specific examples of vectors for yeast hosts include YEp13 and YCp50; specific examples of vectors for plant host cells include pBI121 and pBI101; and specific examples of vectors for animal host cells include pcDNA1 and pcDNA1/Amp.

The transformant of the present invention can be obtained by transfecting host cells with the vector. Examples of methods for transfecting bacterial cells with the recombinant DNA include a method using calcium ions and an electroporation method. Examples of methods for transfecting yeast cells with the recombinant DNA include an electroporation method, a spheroplast method, and a lithium acetate method. Examples of methods for transfecting plant cells with the

recombinant DNA include an *Agrobacterium* infection method, a particle gun method, and a polyethylene glycol method. Examples of methods for transfecting animal cells with the recombinant DNA include an electroporation method and a calcium phosphate method.

An enzyme variant of the present invention can be produced by culturing the aforementioned transformant on a medium to express and accumulate the enzyme variant of the present invention in the cultured cells or the culture supernatant, and collecting the enzyme variant from the culture. Thus, the "culture" herein refers to a culture liquid containing cells or the cultured cells which are obtained by culturing the transformant on a medium. The transformant can be cultured on a medium in accordance with common methods for culturing host cells. Examples of media for culturing transformants of bacteria hosts such as *E. coli* include complete media and synthetic media such as LB medium and M9 medium. Then, the cells are aerobically cultured at a temperature of 20° C. to 40° C. to accumulate the enzyme variant of the present invention therein and the enzyme variant is then recovered.

The enzyme variant of the present invention can be purified by centrifuging the culture obtained by the above culturing method to recover the product (cells are disrupted by a sonicator or the like), followed by performing one or an appropriate combination of affinity chromatography, cation- or anion-exchange chromatography, gel filtration, and the like.

Whether the purified product is the target enzyme can be confirmed by common methods such as SDS polyacrylamide gel electrophoresis and western blotting. Thus, the "purification" of the culture of the transformant in the present invention refers to treatment for removing contaminants other than the target enzyme without losing the enzyme activity.

The enzyme-containing product of the present invention is obtained by purifying the culture of the transformant. Examples of the enzyme-containing product include a cell-free extract obtainable by disrupting cells, an enzyme solution obtained by purification, and a freeze-dried product of the enzyme solution.

A method for producing NAD(P)⁺ from NAD(P)H using the NAD(P)H oxidase variant is also included in the present invention. NAD(P)H (reduced form) is basically generated as a result of, but not limited to, the reduction of NAD(P)⁺ which is a side reaction of the oxidation of a compound catalyzed by an oxidoreductase that recognizes an NAD(P) coenzyme. For example, a case where NAD(P)⁺ is reduced to NAD(P)H by a redox catalyst rather than enzymes is also included in the present invention.

The method for producing NAD(P)⁺ from NAD(P)H using the NAD(P)H oxidase variant according to the present invention can be used for reaction systems involving oxidoreductases (dehydrogenases) with nicotinamide adenine dinucleotide as a coenzyme. The method of the present invention enables NADH/NADPH (reduced form) produced in such a reaction system to be converted and regenerated into NAD⁺/NADP⁺ (oxidized form) by the NAD(P)H oxidase variant.

Namely, the present invention provides water-forming NADH oxidase variants that can be used in combination with oxidoreductases that use NAD(P)⁺ as a coenzyme. Here, the "variants that can be used in combination" means "variants intended to be used in combination". For example, the oxi-

doreductase that uses NAD(P)⁺ as a coenzyme can be used before, during, and/or after using the water-forming NAD(P)H oxidase variant. Preferably, the oxidoreductase that uses NAD(P)⁺ as a coenzyme and the water-forming NADH oxidase variant are simultaneously contacted with their substrates.

Oxidoreductases are enzymes classified as EC 1 and represented by EC.1.X.X.X (X is arbitrary). Oxidoreductases that utilize as a coenzyme NAD⁺/NADP⁺ (oxidized form) regenerated by the present invention are represented by EC.1.X.1.X. Thus, for example, a method for producing an alcohol derivative or a hydroxy acid derivative using an oxidoreductase represented by EC.1.1.1.X and the NAD(P)⁺ regeneration system in the present invention is included in the present invention.

Likewise, the production of an amino acid derivative or a primary amine derivative using an oxidoreductase variant represented by EC.1.4.1.X and the NAD(P)⁺ regeneration system in the present invention is also included in the present invention. The term "derivative" refers to a compound obtained by small structural modification of a certain compound. A compound obtained by substituting a hydrogen atom or a specific group of an original compound by another atom or another group is understood to be a derivative of the original compound.

Oxidoreductases that use nicotinamide adenine dinucleotide as a coenzyme can act on other various compounds such as hydrocarbon chains, nitrogen-containing compounds, and sulfur-containing compounds as substrates, and the types of these oxidoreductases are not limited in the present invention, provided that they are used in combination with the NAD(P)⁺ regeneration system in the present invention.

The NAD(P)H oxidase variant can also be used in a reaction (optical resolution) for producing an optically active compound with a high enantiomeric excess from an enantiomer mixture by stereoselective oxidation catalyzed by the oxidoreductase. The optically active compound with a high enantiomeric excess to be produced is not particularly limited and the NAD(P)H oxidase variant can be used for the production of any optically active compound.

Examples of the "enantiomer mixture" include compounds having an asymmetric carbon to which a hydroxyl, amino, or formyl group, which are oxidizable by dehydrogenases, is attached. Specific examples thereof include alcohol derivatives such as diol derivatives, hydroxy acid derivatives, and amino acid derivatives. More specifically, acyclic 1,2-diols, β -hydroxycarboxylic acids, 2-amino alcohols, non-natural amino acids, and the like are mentioned.

The phrase "high enantiomeric excess" means that the ratio of a target enantiomer in a mixture with the other enantiomer is at least 70 mol %, preferably at least about 90 mol %, and more preferably at least about 95 mol %.

The reaction conditions for use of an NAD(P)H oxidase variant obtained by the present invention depend on a substrate used, an oxidoreductase used in combination, and the like. The reaction is generally performed at a temperature of about 4° C. to 80° C., preferably about 10° C. to 50° C., and at a pH of about 4.0 to 10.0, preferably about 5.0 to 9.0. In the case that the present invention is applied as an NAD(P)⁺ regeneration system, the NAD(P)⁺ concentration is, but is not limited to, about 0.00001 to 1 mol % (w/v), and preferably about 0.00001 to 0.1 mol % (w/v) of the substrate that is catalytically oxidized by the oxidoreductase used in combination.

Since the NAD(P)H oxidase variant obtained in the present invention needs oxygen to catalyze the reaction, the reaction is preferably performed in the presence of air or relatively

pure oxygen. In order to accelerate dissolution of oxygen into the reaction liquid, the reaction is preferably performed under shaking or agitation. Moreover, if the reaction is performed at a pressure higher than atmospheric pressure, the solubility of oxygen in the reaction liquid may be increased, thereby improving the reaction efficiency.

The NAD(P)H oxidase variant may be used as a completely or partially purified enzyme variant. Alternatively, a culture of a microorganism capable of producing the enzyme variant or a processed product thereof may be used. The term “culture” refers to a culture liquid including cells or the cultured cells, and the term “processed product” refers to, for example, a crude extract, freeze-dried cells, acetone-dried cells, or disrupted cells thereof, or a mixture of the foregoing. Moreover, the enzyme itself or the cells themselves may be immobilized by known methods (e.g. cross-linking, physical adsorption, entrapment) before use.

For the reaction, it is not necessary to separately culture microorganisms which respectively express the NAD(P)H oxidase variant and the oxidoreductase that is used in combination if a culture of a transformant microorganism obtained by co-transfection of host cells for the expression of both enzymes or a processed product thereof is used.

Also in the case of using a microorganism that is transformed to co-express both enzymes in the same cell, NAD(P)⁺ in the microorganism cells can be used to perform the reaction. Therefore, there is no need for externally adding another NAD(P)⁺ or the amount of NAD(P)⁺ added can be remarkably reduced. Such a transformant can be produced by incorporating both a DNA encoding the water-forming NADH oxidase variant and a DNA encoding the oxidoreductase used in combination in the same vector, and then transfecting host cells with the vector, or by incorporating these two DNAs into two vectors of different incompatibility groups, respectively, and then co-transfecting host cells with these two vectors.

The following description is offered to illustrate the present invention in more detail by way of Examples, which are by no means intended to limit the scope of the present invention.

EXAMPLES

Example 1

Three-Dimensional Structure Modeling of NADH Oxidase Derived from *Streptococcus mutans*

The program BLAST was used to search sequences highly homologous with the amino acid sequence of SEQ ID No:1. The program used for the search was blastp and the searched database was pdb (aa_db, with the proviso that the database contains all redundant sequences). Multiple amino acid sequence alignments of the amino acid sequence of SEQ ID No:1 and various highly homologous amino acid sequences found by the search were constructed using the program ClustalX. Next, three-dimensional structural alignment is performed on these proteins whose three-dimensional structures are known by using the three-dimensional graphics program Swiss-PDBViewer and the three-dimensional structure comparison/similar structure search server VAST

Search, and then the multiple alignments obtained beforehand based on the amino acid sequences alone were modified based on the similarity between the three-dimensional structures. A three-dimensional structure (PDB code: 2NPX) presumed to have high similarity was selected as a template protein for molecular modeling, based on the resulting sequence alignments. The complex of this template protein with the coenzyme bound thereto was displayed on the program Swiss PDB-Viewer, and subjected to substitution of amino acid residues to correspond to the amino acid sequence (SEQ ID No:1) of the enzyme, based on the sequence alignments. The inserted and deleted sites were replaced by the most suitable similar substructures searched from PDB, whereby a three-dimensional structure model was constructed.

In the manner described above, His-11, Leu-42, Gly-43, Gly-45, Met-46, Tyr-62, and Ala-312 were identified as sites for mutations that can appropriately protect the thiol group of the cysteine residue of the catalytic active site from contact with oxygen. Since His-11 and Tyr-62 are also catalytic residues and mutations at Gly-43 and Gly-45 may largely change the main chain structure, amino acid substitutions at other sites than these were designed.

Tyr-172 was identified as a site for a mutation that can remove the steric hindrance of the NADH-binding site, and an amino acid substitution at this site was designed.

Asn-96, Thr-196, and Phe-371 were identified as sites for mutations that contribute to stabilization of the three-dimensional structure of the enzyme in terms of free energy. Free energy differences between the wild-type and various variants were calculated by computational screening using the program Shrike (JP-A 2001-184381), and amino acid mutations were then designed based on the effect of each amino acid substitution on the free energy difference.

Proteins containing these mutations, that is, water-forming NADH oxidase variants derived from *Streptococcus mutans* are shown as SEQ ID Nos:2 and 4 to 19. The amino acid sequence of SEQ ID No:2 corresponds to the L42M variant; SEQ ID No:4 corresponds to the M46I variant; SEQ ID No:5 corresponds to the N96H variant; SEQ ID No:6 corresponds to the N96R variant; SEQ ID No:7 corresponds to the Y172A variant; SEQ ID No:8 corresponds to the Y172S variant; SEQ ID No:9 corresponds to the T196H variant; SEQ ID No:10 corresponds to the A312I variant; SEQ ID No:11 corresponds to the F371A variant; SEQ ID No:12 corresponds to the F371E variant; SEQ ID No:13 corresponds to the F371V variant; SEQ ID No:14 corresponds to the F371I variant; SEQ ID No:15 corresponds to the F371S variant; SEQ ID No:16 corresponds to the F371T variant; SEQ ID No:17 corresponds to the F371Y variant; SEQ ID No:18 corresponds to the N96R/T196H/F371A variant; and SEQ ID No:19 corresponds to the M46I/N96R/T196H/F371A variant.

Example 2

Construction of Recombinant Vector Containing NADH Oxidase Gene and Preparation of Recombinant *E. coli*

In order to obtain *E. coli* that expresses the water-forming NADH oxidase derived from *Streptococcus mutans*, a recom-

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binant vector for transformation was constructed by the method described in Patent Literature 3. *E. coli* HB101 (Takara Inc.) was transformed with the obtained recombinant vector (pNTNX). As a result, recombinant *E. coli* HB101 (pNTNX) was obtained. The DNA sequence encoding the wild-type NADH oxidase is shown as SEQ ID No:20.

Example 3

Construction of Recombinant Vectors Containing NADH Oxidase Variant Genes and Preparation of Recombinant *E. coli*

Recombinant plasmids respectively containing the NADH oxidase variant genes were obtained by quick change mutagenesis using a pair of synthetic primers designed to introduce mutation(s) at the target site(s) in the DNA sequence encoding the NADH oxidase and the recombinant plasmid pNTNX as a template. The quick change mutagenesis was performed using the QuickChange Site-Directed Mutagenesis Kit (Stratagene Corp.) in accordance with the attached protocol. By way of example, the used pairs of synthetic primers and the resulting variants are described below. For the NADH oxidase variant of SEQ ID No:2 (coding DNA sequence: SEQ ID No:21) including the mutation L42M, a recombinant vector (pNTNX-L042M) for the NADH oxidase L42M variant was obtained by quick change mutagenesis using two synthetic primers of SEQ ID Nos:22 and 23. Appropriate pairs of synthetic primers were designed and used in the same manner to prepare recombinant vectors of SEQ ID Nos:4 to 19 for the respective NADH oxidase variants. For preparation of the variants including multiple mutations of SEQ ID Nos:18 and 19, pNTNX into which a mutation had been introduced was used as a template recombinant plasmid and another mutation was introduced thereto by quick change mutagenesis. The recombinant vectors respectively containing the NADH oxidase variant genes were used to transform *E. coli* HB101 in the same manner as in Example 1, whereby various recombinant *E. coli* cells were obtained.

Example 4

Expression of NADH Oxidase in Recombinant *E. coli*

The various recombinant *E. coli* HB101 cells obtained in Examples 2 and 3 were respectively inoculated on semisyn-

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thetic media (1.5% (w/v) glycerin, 0.3% (w/v) yeast extract, 0.6% (w/v) Na₂HPO₄, 0.3% (w/v) KH₂HPO₄, 0.2% (w/v) NaCl, 0.5% (w/v) MgSO₄·7H₂O, 100 µg/ml ampicillin, pH 7.2), and grown at 37° C. for 38 hours. After collecting cells and removing the culture supernatant from each of the cultures, the residue was suspended in a buffer (50 mM potassium phosphate, pH 7.0) in an amount equivalent to that of the medium and ultrasonically disrupted to provide a cell-free extract. All of the enzyme variant-containing cell-free extracts were found to have NADH oxidase activity under the following measurement conditions.

[Measurement Conditions for NADH Oxidase Activity]

To 0.95 mL of a reaction liquid containing 0.17 mM NADH, 0.2 mM EDTA, and 0.02 mM FAD in a 50 mM potassium phosphate buffer (pH 7.0) was added 0.05 mL of the enzyme liquid (and optionally diluted with the buffer). The mixture was measured at a constant temperature (25° C.) for decrease in absorbance at a wavelength of 340 nm. Under these reaction conditions, one Unit was defined as the enzyme activity that oxidizes 1 µmol of NADH to NAD⁺ for one minute.

Example 5

Stability of Water-Forming NADH Oxidase Variant in the Presence of Oxygen

The HB101 cell-free extracts respectively containing the wild-type water-forming NADH oxidase (control) and the water-forming NADH oxidase variants prepared in Example 4 were diluted with the potassium phosphate buffer to adjust the decrease in absorbance at 340 nm for one minute to about 0.1 to 0.4, and incubated for a predetermined period of time at a constant temperature of 30° C. or 40° C. The enzyme activity was measured before and after the incubation, and the remaining enzyme activity (%) was calculated for the enzymes. The measurements were performed in several runs, and the wild-type water-forming NADH oxidase was used as a control in each run. Table 1 shows the results. The measurements were basically performed under oxygen supply, and the effect of agitation by a stirrer was also investigated in the final measurement.

TABLE 1

Measurement	Wild	N96R	N96H	T196H	M46I			
30° C./18 hours	37%	49%	51%	53%	61%			
40° C./8 hours	14%	25%	23%	27%	53%			
Measurement	Wild	F371S	F371V	F371A	F371I	F371Y	F371E	F371T
30° C./19 hours	33%	44%	40%	48%	51%	42%	43%	50%
40° C./8 hours	17%	34%	32%	42%	43%	35%	32%	43%
Measurement	Wild	N96R/T196H/F371A			M46I/N96R/T196H/F371A			
30° C./19 hours	33%	53%			80%			
Measurement	Wild	Y172S	Y172A					
40° C./8 hours	13%	34%	24%					
Measurement	Wild	L42M	A312I	Y172S	M46I			
25° C./6 hours, agitation (-)	54%	78%	76%	88%	92%			
25° C./6 hours, agitation (+)	29%	60%	73%	44%	56%			

The water-forming NADH oxidase variants maintained higher enzyme activity than that of the wild-type at 30° C. and also maintained high enzyme activity at 40° C. Although the remaining enzyme activity was reduced by agitation with

incubation at the same temperature condition for the same period of time, the fact remains true that the variants maintained higher enzyme activity compared with the wild-type.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 24

<210> SEQ ID NO 1

<211> LENGTH: 457

<212> TYPE: PRT

<213> ORGANISM: Streptococcus mutans

<400> SEQUENCE: 1

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Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
1           5           10           15

Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
          20           25           30

Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
          35           40           45

Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
          50           55           60

Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
65           70           75           80

Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
          85           90           95

Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
          100          105          110

Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
          115          120          125

Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
          130          135          140

Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
          145          150          155          160

Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
          165          170          175

Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
          180          185          190

Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
          195          200          205

Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
          210          215          220

Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
          225          230          235          240

Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
          245          250          255

Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
          260          265          270

Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
          275          280          285

Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
          290          295          300

Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
          305          310          315          320

Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
          325          330          335

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Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
 340 345 350

Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
 355 360 365

Thr Ala Phe Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
 370 375 380

Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
 385 390 395 400

Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
 405 410 415

Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
 420 425 430

Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
 435 440 445

Met Thr Lys Ala Ala Leu Lys Ala Lys
 450 455

<210> SEQ ID NO 2
 <211> LENGTH: 457
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 2

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
 1 5 10 15

Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
 20 25 30

Phe Asp Gln Asn Ser Asn Ile Ser Phe Met Gly Cys Gly Met Ala Leu
 35 40 45

Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
 50 55 60

Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
 65 70 75 80

Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
 85 90 95

Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
 100 105 110

Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
 115 120 125

Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
 130 135 140

Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
 145 150 155 160

Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
 165 170 175

Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
 180 185 190

Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
 195 200 205

Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
 210 215 220

Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
 225 230 235 240

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Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
 245 250 255
 Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
 260 265 270
 Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
 275 280 285
 Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
 290 295 300
 Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
 305 310 315 320
 Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
 325 330 335
 Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
 340 345 350
 Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
 355 360 365
 Thr Ala Phe Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
 370 375 380
 Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
 385 390 395 400
 Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
 405 410 415
 Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
 420 425 430
 Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
 435 440 445
 Met Thr Lys Ala Ala Leu Lys Ala Lys
 450 455

<210> SEQ ID NO 3
 <211> LENGTH: 457
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 3

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
 1 5 10 15
 Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
 20 25 30
 Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Ala Ala Leu
 35 40 45
 Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
 50 55 60
 Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
 65 70 75 80
 Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
 85 90 95
 Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
 100 105 110
 Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
 115 120 125
 Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
 130 135 140

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Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
 145 150 155 160
 Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
 165 170 175
 Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
 180 185 190
 Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
 195 200 205
 Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
 210 215 220
 Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
 225 230 235 240
 Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
 245 250 255
 Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
 260 265 270
 Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
 275 280 285
 Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
 290 295 300
 Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
 305 310 315 320
 Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
 325 330 335
 Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
 340 345 350
 Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
 355 360 365
 Thr Ala Phe Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
 370 375 380
 Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
 385 390 395 400
 Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
 405 410 415
 Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
 420 425 430
 Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
 435 440 445
 Met Thr Lys Ala Ala Leu Lys Ala Lys
 450 455

<210> SEQ ID NO 4
 <211> LENGTH: 457
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 4

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
 1 5 10 15
 Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
 20 25 30
 Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Ile Ala Leu
 35 40 45

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<210> SEQ ID NO 5
<211> LENGTH: 457
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 5
Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
1          5          10          15
Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
20          25          30
Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
35          40          45
Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
50          55          60
Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
65          70          75          80
Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val His
85          90          95
Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
100         105         110
Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
115         120         125
Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
130         135         140
Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
145         150         155         160
Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
165         170         175
Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
180         185         190
Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
195         200         205
Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
210         215         220
Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
225         230         235         240
Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
245         250         255
Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
260         265         270
Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
275         280         285
Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
290         295         300
Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
305         310         315         320
Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
325         330         335
Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
340         345         350
Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
355         360         365
Thr Ala Phe Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn

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370	375	380
Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val 385 390 395 400		
Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His 405 410 415		
Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala 420 425 430		
Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr 435 440 445		
Met Thr Lys Ala Ala Leu Lys Ala Lys 450 455		

<210> SEQ ID NO 6

<211> LENGTH: 457

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 6

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala 1 5 10 15		
Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val 20 25 30		
Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu 35 40 45		
Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp 50 55 60		
Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro 65 70 75 80		
Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Arg 85 90 95		
Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly 100 105 110		
Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly 115 120 125		
Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu 130 135 140		
Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln 145 150 155 160		
Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu 165 170 175		
Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp 180 185 190		
Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu 195 200 205		
Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly 210 215 220		
Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val 225 230 235 240		
Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly 245 250 255		
Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg 260 265 270		
Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp		

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275					280					285					
Val	Tyr	Ala	Ile	Gly	Asp	Cys	Ala	Thr	Val	Tyr	Asp	Asn	Ala	Ile	Asn
290					295					300					
Asp	Thr	Asn	Tyr	Ile	Ala	Leu	Ala	Ser	Asn	Ala	Leu	Arg	Ser	Gly	Ile
305					310					315					320
Val	Ala	Gly	His	Asn	Ala	Ala	Gly	His	Lys	Leu	Glu	Ser	Leu	Gly	Val
				325					330					335	
Gln	Gly	Ser	Asn	Gly	Ile	Ser	Ile	Phe	Gly	Leu	Asn	Met	Val	Ser	Thr
			340					345					350		
Gly	Leu	Thr	Gln	Glu	Lys	Ala	Lys	Arg	Phe	Gly	Tyr	Asn	Pro	Glu	Val
		355					360					365			
Thr	Ala	Phe	Thr	Asp	Phe	Gln	Lys	Ala	Ser	Phe	Ile	Glu	His	Asp	Asn
	370					375					380				
Tyr	Pro	Val	Thr	Leu	Lys	Ile	Val	Tyr	Asp	Lys	Asp	Ser	Arg	Leu	Val
385					390					395					400
Leu	Gly	Ala	Gln	Met	Ala	Ser	Lys	Glu	Asp	Met	Ser	Met	Gly	Ile	His
				405					410					415	
Met	Phe	Ser	Leu	Ala	Ile	Gln	Glu	Lys	Val	Thr	Ile	Glu	Arg	Leu	Ala
			420					425					430		
Leu	Leu	Asp	Tyr	Phe	Phe	Leu	Pro	His	Phe	Asn	Gln	Pro	Tyr	Asn	Tyr
		435					440					445			
Met	Thr	Lys	Ala	Ala	Leu	Lys	Ala	Lys							
	450					455									

<210> SEQ ID NO 7

<211> LENGTH: 457

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 7

Met	Ser	Lys	Ile	Val	Ile	Val	Gly	Ala	Asn	His	Ala	Gly	Thr	Ala	Ala
1				5					10					15	
Ile	Asn	Thr	Ile	Leu	Asp	Asn	Tyr	Gly	Ser	Glu	Asn	Glu	Val	Val	Val
			20					25					30		
Phe	Asp	Gln	Asn	Ser	Asn	Ile	Ser	Phe	Leu	Gly	Cys	Gly	Met	Ala	Leu
		35					40					45			
Trp	Ile	Gly	Lys	Gln	Ile	Ser	Gly	Pro	Gln	Gly	Leu	Phe	Tyr	Ala	Asp
		50					55					60			
Lys	Glu	Ser	Leu	Glu	Ala	Lys	Gly	Ala	Lys	Ile	Tyr	Met	Glu	Ser	Pro
65						70					75				80
Val	Thr	Ala	Ile	Asp	Tyr	Asp	Ala	Lys	Arg	Val	Thr	Ala	Leu	Val	Asn
				85					90					95	
Gly	Gln	Glu	His	Val	Glu	Ser	Tyr	Glu	Lys	Leu	Ile	Leu	Ala	Thr	Gly
			100					105					110		
Ser	Thr	Pro	Ile	Leu	Pro	Pro	Ile	Lys	Gly	Ala	Ala	Ile	Lys	Glu	Gly
		115					120					125			
Ser	Arg	Asp	Phe	Glu	Ala	Thr	Leu	Lys	Asn	Leu	Gln	Phe	Val	Lys	Leu
		130				135					140				
Tyr	Gln	Asn	Ala	Glu	Asp	Val	Ile	Asn	Lys	Leu	Gln	Asp	Lys	Ser	Gln
145						150					155				160
Asn	Leu	Asn	Arg	Ile	Ala	Val	Val	Gly	Ala	Gly	Ala	Ile	Gly	Val	Glu
			165						170					175	
Leu	Ala	Glu	Ala	Phe	Lys	Arg	Leu	Gly	Lys	Glu	Val	Ile	Leu	Ile	Asp

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180					185					190					
Val	Val	Asp	Thr	Cys	Leu	Ala	Gly	Tyr	Tyr	Asp	Gln	Asp	Leu	Ser	Glu
		195					200					205			
Met	Met	Arg	Gln	Asn	Leu	Glu	Asp	His	Gly	Ile	Glu	Leu	Ala	Phe	Gly
		210					215					220			
Glu	Thr	Val	Lys	Ala	Ile	Glu	Gly	Asp	Gly	Lys	Val	Glu	Arg	Ile	Val
		225					230					235			
Thr	Asp	Lys	Ala	Ser	His	Asp	Val	Asp	Met	Val	Ile	Leu	Ala	Val	Gly
				245					250					255	
Phe	Arg	Pro	Asn	Thr	Ala	Leu	Gly	Asn	Ala	Lys	Leu	Lys	Thr	Phe	Arg
			260					265					270		
Asn	Gly	Ala	Phe	Leu	Val	Asp	Lys	Lys	Gln	Glu	Thr	Ser	Ile	Pro	Asp
		275					280					285			
Val	Tyr	Ala	Ile	Gly	Asp	Cys	Ala	Thr	Val	Tyr	Asp	Asn	Ala	Ile	Asn
		290					295					300			
Asp	Thr	Asn	Tyr	Ile	Ala	Leu	Ala	Ser	Asn	Ala	Leu	Arg	Ser	Gly	Ile
		305					310					315			320
Val	Ala	Gly	His	Asn	Ala	Ala	Gly	His	Lys	Leu	Glu	Ser	Leu	Gly	Val
				325					330					335	
Gln	Gly	Ser	Asn	Gly	Ile	Ser	Ile	Phe	Gly	Leu	Asn	Met	Val	Ser	Thr
			340					345					350		
Gly	Leu	Thr	Gln	Glu	Lys	Ala	Lys	Arg	Phe	Gly	Tyr	Asn	Pro	Glu	Val
		355					360					365			
Thr	Ala	Phe	Thr	Asp	Phe	Gln	Lys	Ala	Ser	Phe	Ile	Glu	His	Asp	Asn
		370					375					380			
Tyr	Pro	Val	Thr	Leu	Lys	Ile	Val	Tyr	Asp	Lys	Asp	Ser	Arg	Leu	Val
		385					390					395			400
Leu	Gly	Ala	Gln	Met	Ala	Ser	Lys	Glu	Asp	Met	Ser	Met	Gly	Ile	His
				405					410					415	
Met	Phe	Ser	Leu	Ala	Ile	Gln	Glu	Lys	Val	Thr	Ile	Glu	Arg	Leu	Ala
			420					425					430		
Leu	Leu	Asp	Tyr	Phe	Phe	Leu	Pro	His	Phe	Asn	Gln	Pro	Tyr	Asn	Tyr
		435					440					445			
Met	Thr	Lys	Ala	Ala	Leu	Lys	Ala	Lys							
		450					455								

<210> SEQ ID NO 8

<211> LENGTH: 457

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 8

Met	Ser	Lys	Ile	Val	Ile	Val	Gly	Ala	Asn	His	Ala	Gly	Thr	Ala	Ala
1				5					10					15	
Ile	Asn	Thr	Ile	Leu	Asp	Asn	Tyr	Gly	Ser	Glu	Asn	Glu	Val	Val	Val
			20					25					30		
Phe	Asp	Gln	Asn	Ser	Asn	Ile	Ser	Phe	Leu	Gly	Cys	Gly	Met	Ala	Leu
		35					40					45			
Trp	Ile	Gly	Lys	Gln	Ile	Ser	Gly	Pro	Gln	Gly	Leu	Phe	Tyr	Ala	Asp
		50					55					60			
Lys	Glu	Ser	Leu	Glu	Ala	Lys	Gly	Ala	Lys	Ile	Tyr	Met	Glu	Ser	Pro
		65					70					75			80
Val	Thr	Ala	Ile	Asp	Tyr	Asp	Ala	Lys	Arg	Val	Thr	Ala	Leu	Val	Asn

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85					90					95					
Gly	Gln	Glu	His	Val	Glu	Ser	Tyr	Glu	Lys	Leu	Ile	Leu	Ala	Thr	Gly
			100					105					110		
Ser	Thr	Pro	Ile	Leu	Pro	Pro	Ile	Lys	Gly	Ala	Ala	Ile	Lys	Glu	Gly
		115					120					125			
Ser	Arg	Asp	Phe	Glu	Ala	Thr	Leu	Lys	Asn	Leu	Gln	Phe	Val	Lys	Leu
	130					135					140				
Tyr	Gln	Asn	Ala	Glu	Asp	Val	Ile	Asn	Lys	Leu	Gln	Asp	Lys	Ser	Gln
145				150						155					160
Asn	Leu	Asn	Arg	Ile	Ala	Val	Val	Gly	Ala	Gly	Ser	Ile	Gly	Val	Glu
			165					170					175		
Leu	Ala	Glu	Ala	Phe	Lys	Arg	Leu	Gly	Lys	Glu	Val	Ile	Leu	Ile	Asp
		180						185					190		
Val	Val	Asp	Thr	Cys	Leu	Ala	Gly	Tyr	Tyr	Asp	Gln	Asp	Leu	Ser	Glu
		195					200					205			
Met	Met	Arg	Gln	Asn	Leu	Glu	Asp	His	Gly	Ile	Glu	Leu	Ala	Phe	Gly
	210					215					220				
Glu	Thr	Val	Lys	Ala	Ile	Glu	Gly	Asp	Gly	Lys	Val	Glu	Arg	Ile	Val
225				230						235					240
Thr	Asp	Lys	Ala	Ser	His	Asp	Val	Asp	Met	Val	Ile	Leu	Ala	Val	Gly
			245						250					255	
Phe	Arg	Pro	Asn	Thr	Ala	Leu	Gly	Asn	Ala	Lys	Leu	Lys	Thr	Phe	Arg
			260					265					270		
Asn	Gly	Ala	Phe	Leu	Val	Asp	Lys	Lys	Gln	Glu	Thr	Ser	Ile	Pro	Asp
	275						280					285			
Val	Tyr	Ala	Ile	Gly	Asp	Cys	Ala	Thr	Val	Tyr	Asp	Asn	Ala	Ile	Asn
	290					295					300				
Asp	Thr	Asn	Tyr	Ile	Ala	Leu	Ala	Ser	Asn	Ala	Leu	Arg	Ser	Gly	Ile
305				310					315					320	
Val	Ala	Gly	His	Asn	Ala	Ala	Gly	His	Lys	Leu	Glu	Ser	Leu	Gly	Val
			325						330					335	
Gln	Gly	Ser	Asn	Gly	Ile	Ser	Ile	Phe	Gly	Leu	Asn	Met	Val	Ser	Thr
			340					345					350		
Gly	Leu	Thr	Gln	Glu	Lys	Ala	Lys	Arg	Phe	Gly	Tyr	Asn	Pro	Glu	Val
		355					360					365			
Thr	Ala	Phe	Thr	Asp	Phe	Gln	Lys	Ala	Ser	Phe	Ile	Glu	His	Asp	Asn
	370					375					380				
Tyr	Pro	Val	Thr	Leu	Lys	Ile	Val	Tyr	Asp	Lys	Asp	Ser	Arg	Leu	Val
385				390						395				400	
Leu	Gly	Ala	Gln	Met	Ala	Ser	Lys	Glu	Asp	Met	Ser	Met	Gly	Ile	His
			405						410				415		
Met	Phe	Ser	Leu	Ala	Ile	Gln	Glu	Lys	Val	Thr	Ile	Glu	Arg	Leu	Ala
			420					425					430		
Leu	Leu	Asp	Tyr	Phe	Phe	Leu	Pro	His	Phe	Asn	Gln	Pro	Tyr	Asn	Tyr
		435					440					445			
Met	Thr	Lys	Ala	Ala	Leu	Lys	Ala	Lys							
	450					455									

<210> SEQ ID NO 9

<211> LENGTH: 457

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: NOX mutant

-continued

<400> SEQUENCE: 9

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
 1 5 10 15
 Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
 20 25 30
 Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
 35 40 45
 Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
 50 55 60
 Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
 65 70 75 80
 Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
 85 90 95
 Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
 100 105 110
 Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
 115 120 125
 Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
 130 135 140
 Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
 145 150 155 160
 Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
 165 170 175
 Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
 180 185 190
 Val Val Asp His Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
 195 200 205
 Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
 210 215 220
 Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
 225 230 235 240
 Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
 245 250 255
 Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
 260 265 270
 Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
 275 280 285
 Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
 290 295 300
 Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
 305 310 315 320
 Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
 325 330 335
 Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
 340 345 350
 Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
 355 360 365
 Thr Ala Phe Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
 370 375 380
 Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
 385 390 395 400
 Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
 405 410 415

-continued

Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
 420 425 430

Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
 435 440 445

Met Thr Lys Ala Ala Leu Lys Ala Lys
 450 455

<210> SEQ ID NO 10
 <211> LENGTH: 457
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 10

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
 1 5 10 15

Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
 20 25 30

Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
 35 40 45

Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
 50 55 60

Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
 65 70 75 80

Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
 85 90 95

Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
 100 105 110

Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
 115 120 125

Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
 130 135 140

Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
 145 150 155 160

Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
 165 170 175

Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
 180 185 190

Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
 195 200 205

Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
 210 215 220

Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
 225 230 235 240

Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
 245 250 255

Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
 260 265 270

Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
 275 280 285

Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
 290 295 300

Asp Thr Asn Tyr Ile Ala Leu Ile Ser Asn Ala Leu Arg Ser Gly Ile
 305 310 315 320

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Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
 325 330 335
 Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
 340 345 350
 Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
 355 360 365
 Thr Ala Phe Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
 370 375 380
 Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
 385 390 395 400
 Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
 405 410 415
 Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
 420 425 430
 Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
 435 440 445
 Met Thr Lys Ala Ala Leu Lys Ala Lys
 450 455

<210> SEQ ID NO 11
 <211> LENGTH: 457
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 11

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
 1 5 10 15
 Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
 20 25 30
 Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
 35 40 45
 Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
 50 55 60
 Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
 65 70 75 80
 Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
 85 90 95
 Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
 100 105 110
 Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
 115 120 125
 Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
 130 135 140
 Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
 145 150 155 160
 Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
 165 170 175
 Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
 180 185 190
 Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
 195 200 205
 Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
 210 215 220

-continued

Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
 225 230 235 240
 Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
 245 250 255
 Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
 260 265 270
 Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
 275 280 285
 Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
 290 295 300
 Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
 305 310 315 320
 Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
 325 330 335
 Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
 340 345 350
 Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
 355 360 365
 Thr Ala Ala Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
 370 375 380
 Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
 385 390 395 400
 Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
 405 410 415
 Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
 420 425 430
 Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
 435 440 445
 Met Thr Lys Ala Ala Leu Lys Ala Lys
 450 455

<210> SEQ ID NO 12

<211> LENGTH: 457

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 12

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
 1 5 10 15
 Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
 20 25 30
 Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
 35 40 45
 Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
 50 55 60
 Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
 65 70 75 80
 Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
 85 90 95
 Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
 100 105 110
 Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
 115 120 125

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Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
 130 135 140
 Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
 145 150 155 160
 Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
 165 170 175
 Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
 180 185 190
 Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
 195 200 205
 Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
 210 215 220
 Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
 225 230 235 240
 Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
 245 250 255
 Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
 260 265 270
 Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
 275 280 285
 Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
 290 295 300
 Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
 305 310 315 320
 Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
 325 330 335
 Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
 340 345 350
 Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
 355 360 365
 Thr Ala Glu Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
 370 375 380
 Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
 385 390 395 400
 Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
 405 410 415
 Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
 420 425 430
 Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
 435 440 445
 Met Thr Lys Ala Ala Leu Lys Ala Lys
 450 455

<210> SEQ ID NO 13
 <211> LENGTH: 457
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 13

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
 1 5 10 15
 Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
 20 25 30

-continued

Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
35 40 45
Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
50 55 60
Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
65 70 75 80
Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
85 90 95
Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
100 105 110
Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
115 120 125
Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
130 135 140
Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
145 150 155 160
Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
165 170 175
Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
180 185 190
Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
195 200 205
Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
210 215 220
Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
225 230 235 240
Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
245 250 255
Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
260 265 270
Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
275 280 285
Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
290 295 300
Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
305 310 315 320
Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
325 330 335
Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
340 345 350
Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
355 360 365
Thr Ala Val Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
370 375 380
Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
385 390 395 400
Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
405 410 415
Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
420 425 430
Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
435 440 445

-continued

Met Thr Lys Ala Ala Leu Lys Ala Lys
450 455

<210> SEQ ID NO 14
<211> LENGTH: 457
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 14

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
1 5 10 15
Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
20 25 30
Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
35 40 45
Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
50 55 60
Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
65 70 75 80
Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
85 90 95
Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
100 105 110
Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
115 120 125
Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
130 135 140
Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
145 150 155 160
Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
165 170 175
Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
180 185 190
Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
195 200 205
Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
210 215 220
Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
225 230 235 240
Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
245 250 255
Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
260 265 270
Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
275 280 285
Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
290 295 300
Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
305 310 315 320
Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
325 330 335
Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
340 345 350

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Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
 355 360 365

Thr Ala Ile Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
 370 375 380

Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
 385 390 395 400

Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
 405 410 415

Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
 420 425 430

Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
 435 440 445

Met Thr Lys Ala Ala Leu Lys Ala Lys
 450 455

<210> SEQ ID NO 15
 <211> LENGTH: 457
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 15

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
 1 5 10 15

Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
 20 25 30

Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
 35 40 45

Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
 50 55 60

Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
 65 70 75 80

Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
 85 90 95

Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
 100 105 110

Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
 115 120 125

Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
 130 135 140

Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
 145 150 155 160

Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
 165 170 175

Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
 180 185 190

Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
 195 200 205

Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
 210 215 220

Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
 225 230 235 240

Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
 245 250 255

-continued

Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
 260 265 270
 Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
 275 280 285
 Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
 290 295 300
 Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
 305 310 315 320
 Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
 325 330 335
 Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
 340 345 350
 Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
 355 360 365
 Thr Ala Ser Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
 370 375 380
 Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
 385 390 395 400
 Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
 405 410 415
 Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
 420 425 430
 Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
 435 440 445
 Met Thr Lys Ala Ala Leu Lys Ala Lys
 450 455

<210> SEQ ID NO 16
 <211> LENGTH: 457
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 16

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
 1 5 10 15
 Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
 20 25 30
 Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
 35 40 45
 Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
 50 55 60
 Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
 65 70 75 80
 Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
 85 90 95
 Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
 100 105 110
 Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
 115 120 125
 Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
 130 135 140
 Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
 145 150 155 160

-continued

Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
 65 70 75 80
 Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Asn
 85 90 95
 Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
 100 105 110
 Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
 115 120 125
 Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
 130 135 140
 Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
 145 150 155 160
 Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
 165 170 175
 Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
 180 185 190
 Val Val Asp Thr Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
 195 200 205
 Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
 210 215 220
 Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
 225 230 235 240
 Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
 245 250 255
 Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
 260 265 270
 Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
 275 280 285
 Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
 290 295 300
 Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
 305 310 315 320
 Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
 325 330 335
 Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
 340 345 350
 Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
 355 360 365
 Thr Ala Tyr Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
 370 375 380
 Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val
 385 390 395 400
 Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His
 405 410 415
 Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala
 420 425 430
 Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr
 435 440 445
 Met Thr Lys Ala Ala Leu Lys Ala Lys
 450 455

<210> SEQ ID NO 18

<211> LENGTH: 457

<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 18

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Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala
1           5           10           15
Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val
20           25           30
Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Met Ala Leu
35           40           45
Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp
50           55           60
Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro
65           70           75           80
Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Arg
85           90           95
Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly
100          105          110
Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly
115          120          125
Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu
130          135          140
Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln
145          150          155          160
Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu
165          170          175
Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp
180          185          190
Val Val Asp His Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu
195          200          205
Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly
210          215          220
Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val
225          230          235          240
Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly
245          250          255
Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg
260          265          270
Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp
275          280          285
Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn
290          295          300
Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile
305          310          315          320
Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val
325          330          335
Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr
340          345          350
Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val
355          360          365
Thr Ala Ala Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn
370          375          380
Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val

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385	390	395	400
Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His	405	410	415
Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala	420	425	430
Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr	435	440	445
Met Thr Lys Ala Ala Leu Lys Ala Lys	450	455	

<210> SEQ ID NO 19

<211> LENGTH: 457

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 19

Met Ser Lys Ile Val Ile Val Gly Ala Asn His Ala Gly Thr Ala Ala	1	5	10	15
Ile Asn Thr Ile Leu Asp Asn Tyr Gly Ser Glu Asn Glu Val Val Val	20	25	30	
Phe Asp Gln Asn Ser Asn Ile Ser Phe Leu Gly Cys Gly Ile Ala Leu	35	40	45	
Trp Ile Gly Lys Gln Ile Ser Gly Pro Gln Gly Leu Phe Tyr Ala Asp	50	55	60	
Lys Glu Ser Leu Glu Ala Lys Gly Ala Lys Ile Tyr Met Glu Ser Pro	65	70	75	80
Val Thr Ala Ile Asp Tyr Asp Ala Lys Arg Val Thr Ala Leu Val Arg	85	90	95	
Gly Gln Glu His Val Glu Ser Tyr Glu Lys Leu Ile Leu Ala Thr Gly	100	105	110	
Ser Thr Pro Ile Leu Pro Pro Ile Lys Gly Ala Ala Ile Lys Glu Gly	115	120	125	
Ser Arg Asp Phe Glu Ala Thr Leu Lys Asn Leu Gln Phe Val Lys Leu	130	135	140	
Tyr Gln Asn Ala Glu Asp Val Ile Asn Lys Leu Gln Asp Lys Ser Gln	145	150	155	160
Asn Leu Asn Arg Ile Ala Val Val Gly Ala Gly Tyr Ile Gly Val Glu	165	170	175	
Leu Ala Glu Ala Phe Lys Arg Leu Gly Lys Glu Val Ile Leu Ile Asp	180	185	190	
Val Val Asp His Cys Leu Ala Gly Tyr Tyr Asp Gln Asp Leu Ser Glu	195	200	205	
Met Met Arg Gln Asn Leu Glu Asp His Gly Ile Glu Leu Ala Phe Gly	210	215	220	
Glu Thr Val Lys Ala Ile Glu Gly Asp Gly Lys Val Glu Arg Ile Val	225	230	235	240
Thr Asp Lys Ala Ser His Asp Val Asp Met Val Ile Leu Ala Val Gly	245	250	255	
Phe Arg Pro Asn Thr Ala Leu Gly Asn Ala Lys Leu Lys Thr Phe Arg	260	265	270	
Asn Gly Ala Phe Leu Val Asp Lys Lys Gln Glu Thr Ser Ile Pro Asp	275	280	285	
Val Tyr Ala Ile Gly Asp Cys Ala Thr Val Tyr Asp Asn Ala Ile Asn				

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290	295	300
Asp Thr Asn Tyr Ile Ala Leu Ala Ser Asn Ala Leu Arg Ser Gly Ile 305 310 315 320		
Val Ala Gly His Asn Ala Ala Gly His Lys Leu Glu Ser Leu Gly Val 325 330 335		
Gln Gly Ser Asn Gly Ile Ser Ile Phe Gly Leu Asn Met Val Ser Thr 340 345 350		
Gly Leu Thr Gln Glu Lys Ala Lys Arg Phe Gly Tyr Asn Pro Glu Val 355 360 365		
Thr Ala Ala Thr Asp Phe Gln Lys Ala Ser Phe Ile Glu His Asp Asn 370 375 380		
Tyr Pro Val Thr Leu Lys Ile Val Tyr Asp Lys Asp Ser Arg Leu Val 385 390 395 400		
Leu Gly Ala Gln Met Ala Ser Lys Glu Asp Met Ser Met Gly Ile His 405 410 415		
Met Phe Ser Leu Ala Ile Gln Glu Lys Val Thr Ile Glu Arg Leu Ala 420 425 430		
Leu Leu Asp Tyr Phe Phe Leu Pro His Phe Asn Gln Pro Tyr Asn Tyr 435 440 445		
Met Thr Lys Ala Ala Leu Lys Ala Lys 450 455		

<210> SEQ ID NO 20

<211> LENGTH: 1374

<212> TYPE: DNA

<213> ORGANISM: Streptococcus mutans

<400> SEQUENCE: 20

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ctagataatt acggtagtga aaacgaagtt gtcgtttttg accaaaattc taatatttca      120
ttcttggggt gtggaatggc actttggatt ggaaaacaaa tatcaggccc tcaaggtcct      180
ttttatgctg acaaggaatc gttagaagca aaaggtgcta aaatttatat ggaatcgcca      240
gtgacagcca ttgattatga tgctaagagg gttactgctt tgggtcaatgg tcaagaacat      300
gttgaaagct atgagaagct tattttggca acaggatcaa caccaatcct accacctatc      360
aaaggtgcag ctatcaaaga aggtagtcgt gattttgaag caactttgaa aaatcttcaa      420
tttgtaaata tgtatcaaaa tgcagaagat gttattaata aattacagga taagagtcaa      480
aatctgaatc gtattgctgt tgttgggtgct gggttatattg gtgtagaact tgctgaagcc      540
tttaaacgcc tcggaaaaga agtgattcct attgatgttg ttgatacttg cttagctggg      600
tattatgata aggatctttc agaaatgatg cgtcaaaaatt tggaagatca tggatttgaa      660
ttagcattcg gagaaactgt caaagccatt gaaggtgatg gtaaagtcga acgtattgta      720
actgataaag cgagccatga tgtggatatg gttattttag ctgtcggttt ccgtcctaata      780
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aaacaagaga caagtattcc tgacgtttat gccatcgggc attgcgcgac tgtttatgac      900
aacgctatta atgataccaa ttatattgcc ttagcttcaa acgctcttcg ctcaggtatt      960
gtagctggtc ataatgcagc agggcataaa ttggaatctc ttgggtgtca aggttcaaat     1020
ggatatttcaa tttttggtct caatatgggt tcaactgggt taacacaaga aaaagcaaag     1080
cgttttggct ataatccaga agtcactgca tttacagatt ttcagaaggc tagttttatt     1140
gaacatgata attatcctgt tacacttaaa attgtctatg ataaggatag ccgactgggt     1200

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cttgggtgcac aaatggcatc taaagaagat atgtcaatgg gaattcacat gttttcattg 1260
gctattcagg aaaaagttac cattgaacgt ttagctctac tggactattt ctttcttcct 1320
catttcaatc aaccctataa ttatatgacc aaagcagcat taaaagctaa atga 1374

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<210> SEQ ID NO 21
<211> LENGTH: 1374
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NOX mutant

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<400> SEQUENCE: 21

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ttcatggggt gtggaatggc actttggatt ggaaaaacaaa tatcaggccc tcaaggtcct 180
ttttatgctg acaaggaatc gttagaagca aaaggtgcta aaatttatat ggaatcgcca 240
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gttgaaagct atgagaagct tattttggca acaggatcaa caccaatctt accacctatc 360
aaaggtgcag ctatcaaaga aggtagtcgt gattttgaag caactttgaa aaatcttcaa 420
tttgttaaat tgtatcaaaa tgcagaagat gttattaata aattacagga taagagtcaa 480
aatctgaatc gtattgctgt tgttgggtgct ggttatattg gtgtagaact tgctgaagcc 540
tttaaagccc tcggaaaaga agtgattctt attgatgttg ttgatacttg cttagctggt 600
tattatgatac aggatctttc agaaatgatg cgtcaaaaatt tggaagatca tggattgaa 660
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gaacatgata attatcctgt tacacttaaa attgtctatg ataaggatag ccgactgggt 1200
cttgggtgcac aaatggcatc taaagaagat atgtcaatgg gaattcacat gttttcattg 1260
gctattcagg aaaaagttac cattgaacgt ttagctctac tggactattt ctttcttcct 1320
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<210> SEQ ID NO 22
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic DNA primer

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<400> SEQUENCE: 22

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ctaataatttc attcatgggt tgtggaatg 29

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<210> SEQ ID NO 23
<211> LENGTH: 29
<212> TYPE: DNA

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<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA primer

<400> SEQUENCE: 23

cattccacaa cccatgaatg aaatattag                29

<210> SEQ ID NO 24
<211> LENGTH: 1374
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: NOX mutant

<400> SEQUENCE: 24

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ctagataatt acggtagtga aaacgaagtt gtcgtttttg accaaaattc taatatttca    120
ttcttggggt gtggaatcgc actttggatt ggaaaacaaa tatcaggccc tcaaggtcct    180
ttttatgctg acaaggaatc gttagaagca aaaggtgcta aaatttatat ggaatcgcca    240
gtgacagcca ttgattatga tgctaagagg gttactgctt tggatcaatgg tcaagaacat    300
gttgaaagct atgagaagct tattttggca acaggatcaa caccaatctt accacctatc    360
aaaggtgcag ctatcaaaga aggtagtcgt gattttgaag caactttgaa aaatcttcaa    420
tttgtaaat  tgtatcaaaa tgcagaagat gttattaata aattacagga taagagtcaa    480
aatctgaatc gtattgctgt tgttgggtgct ggttatattg gtgtagaact tgctgaagcc    540
tttaaacgcc tcggaagaaga agtgattctt attgatgttg ttgatacttg cttagctggt    600
tattatgata aggatctttc agaaatgatg cgtcaaaatt tggaagatca tggatttgaa    660
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gtagctggtc ataatgcagc agggcataaa ttggaatctc ttgggtgtca aggttcaaat   1020
ggtatttcaa tttttggtct caatatgggt tcaactgggt taacacaaga aaaagcaaag   1080
cgttttggct ataatccaga agtcactgca tttacagatt ttcagaaggc tagttttatt   1140
gaacatgata attatcctgt tacacttaaa attgtctatg ataaggatag ccgactgggt   1200
cttgggtgcac aatggcatc taaagaagat atgtcaatgg gaattcacat gttttcattg   1260
gctattcagg aaaaagttac cattgaacgt ttagctctac tggactattt cttttctcct   1320
catttcaatc aaccctataa ttatatgacc aaagcagcat taaaagctaa atga         1374

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The invention claimed is:

1. An isolated protein which has NADH oxidase activity or NADPH oxidase activity or both and has improved stability compared to the protein having the amino acid sequence of SEQ ID NO: 1, wherein said protein has an amino acid sequence that has at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1 and further contains at least one amino acid substitution selected from (a) to (g):

(a) a substitution of an amino acid residue at a position corresponding to position 42 of SEQ ID NO: 1 with an amino acid having a side-chain surface area of 100 to 200 Å²;

(b) a substitution of an amino acid residue at a position corresponding to position 46 of SEQ ID NO: 1 with a neutral amino acid having a side-chain surface area of 100 to 150 Å² or an acidic amino acid having a side-chain surface area of 100 to 150 Å²;

(c) a substitution of an amino acid residue at a position corresponding to position 96 of SEQ ID NO: 1 with a basic amino acid;

(d) a substitution of an amino acid residue at a position corresponding to position 172 of SEQ ID NO: 1 with an amino acid having a smaller side-chain surface area than Tyr;

- (e) a substitution of an amino acid residue at a position corresponding to position 196 of SEQ ID NO:1 with a basic amino acid;
- (f) a substitution of an amino acid residue at a position corresponding to position 312 of SEQ ID NO: 1 with an amino acid having a larger side-chain surface area than Ala; and
- (g) a substitution of an amino acid residue at a position corresponding to position 371 of SEQ ID NO: 1 with an aliphatic amino acid, an acidic amino acid, or an amino acid having a hydroxyl group-bearing side chain.
2. The protein according to claim 1, wherein the amino acid sequence contains at least one amino acid substitution selected from (a) to (g):
- (a) a substitution of an amino acid residue at a position corresponding to position 42 of SEQ ID NO:1 with Met;
- (b) a substitution of an amino acid residue at a position corresponding to position 46 of SEQ ID NO:1 with Ile;
- (c) a substitution of an amino acid residue at a position corresponding to position 96 of SEQ ID NO:1 with Arg or His;
- (d) a substitution of an amino acid residue at a position corresponding to position 172 of SEQ ID NO:1 with Ala or Ser;
- (e) a substitution of an amino acid residue at a position corresponding to position 197 of SEQ ID NO:1 with His;
- (f) a substitution of an amino acid residue at a position corresponding to position 312 of SEQ ID NO:1 with Ile; and
- (g) a substitution of an amino acid residue at a position corresponding to position 371 of SEQ ID NO: 1 with Ala, Val, Ile, Glu, Ser, Thr, or Tyr.
3. An isolated protein which comprises an amino acid sequence identical to SEQ ID NO: 1 except for one or more substitutions selected from the group consisting of:
- (a) a substitution of Leu at position 42 of SEQ ID NO: 1 with an amino acid having a side-chain surface area of 100 to 200 Å²;
- (b) a substitution of Met at position 46 of SEQ ID NO: 1 with a neutral amino acid having a side-chain surface area of no more than 150 Å² or an acidic amino acid having a side-chain surface area of no more than 150 Å²;
- (c) a substitution of Asn at position 96 of SEQ ID NO: 1 with a basic amino acid;
- (d) a substitution of Tyr at position 172 of SEQ ID NO: 1 with an amino acid having a smaller side-chain surface area than Tyr;
- (e) a substitution of Thr at position 196 of SEQ ID NO: 1 with a basic amino acid;
- (f) a substitution of Ala at position 312 with an amino acid having a larger side-chain surface area than Ala; and
- (g) a substitution of Phe at position 371 of SEQ ID NO: 1 with an aliphatic amino acid, an acidic amino acid, or an amino acid having a hydroxyl group-bearing side chain.
4. The protein according to claim 3 wherein said protein comprises an amino acid sequence identical to SEQ ID NO: 1 except for one or more substitutions selected from the group consisting of:

- (a) a substitution of Leu at position 42 of SEQ ID NO: 1 with Met;
- (b) a substitution of Met at position 46 of SEQ ID NO: 1 with Ile;
- (c) a substitution of Asn at position 96 of SEQ ID NO: 1 with Arg or His;
- (d) a substitution of Tyr at position 172 of SEQ ID NO: 1 with Ala or Ser;
- (e) a substitution of Thr at position 196 of SEQ ID NO: 1 with His;
- (f) a substitution of Ala at position 312 with Ile; and
- (g) a substitution of Phe at position 371 of SEQ ID NO: 1 with Ala, Val, Ile, Glu, Ser, Thr, or Tyr.
5. The protein according to claim 4, wherein the protein has an amino acid sequence selected from the amino acid sequences of SEQ ID NO:2 and 4 to 19.
6. A product comprising a protein, wherein said product is obtained by processing a culture of a host cell transformed with a vector which encodes said protein, wherein said protein has NADH oxidase activity or NADPH oxidase activity or both and has improved stability compared to the protein having the amino acid sequence of SEQ ID NO: 1, and wherein said protein has an amino acid sequence that has at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1 and further contains at least one amino acid substitution selected from (a) to (g):
- (a) a substitution of an amino acid residue at a position corresponding to position 42 of SEQ ID NO: 1 with an amino acid having a side-chain surface area of 100 to 200 Å²;
- (b) a substitution of an amino acid residue at a position corresponding to position 46 of SEQ ID NO: 1 with a neutral amino acid having a side-chain surface area of 100 to 150 Å² or an acidic amino acid having a side-chain surface area of 100 to 150 Å²;
- (c) a substitution of an amino acid residue at a position corresponding to position 96 of SEQ ID NO: 1 with a basic amino acid;
- (d) a substitution of an amino acid residue at a position corresponding to position 172 of SEQ ID NO: 1 with an amino acid having a smaller side-chain surface area than Tyr;
- (e) a substitution of an amino acid residue at a position corresponding to position 196 of SEQ ID NO:1 with a basic amino acid;
- (f) a substitution of an amino acid residue at a position corresponding to position 312 of SEQ ID NO: 1 with an amino acid having a larger side-chain surface area than Ala; and
- (g) a substitution of an amino acid residue at a position corresponding to position 371 of SEQ ID NO: 1 with an aliphatic amino acid, an acidic amino acid, or an amino acid having a hydroxyl group-bearing side chain.